Embryonic Stem Cell Derived Mesenchymal Stem Cells

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AND PUBLIC HEALTH

Human Embryonic Stem Cells (hESCs)

Embryonic Stem Cell Lines Derived from Human Blastocysts

James A. Thomson,* Joseph Itskovitz-Eldor, Sander S. Shapiro, Michelle A. Waknitz, Jennifer J. Swiergiel, Vivienne S. Marshall, Jeffrey M. Jones

Human blastocyst-derived, pluripotent cell lines are described that have normal karyotypes, express high levels of telomerase activity, and express cell surface markers that characterize primate embryonic stem cells but do not characterize other early lineages. After undifferentiated proliferation in vitro for 4 to 5 months, these cells still maintained the developmental potential to form trophoblast and derivatives of all three embryonic germ layers, including gut epithelium (endoderm); cartilage, bone, smooth muscle, and striated muscle (mesoderm); and neural epithelium, embryonic ganglia, and stratified squamous epithelium (ectoderm). These cell lines should be useful in human developmental biology, drug discovery, and transplantation medicine.

Embryonic stem (ES) cells are derived from totipotent cells of the early mammalian embryo and are capable of unlimited, undifferentiated proliferation in vitro (*1*, *2*). In chimeras with intact embryos, mouse ES cells contribute to a wide range of adult tissues, including germ cells, providing a powerful approach for introducing specific genetic changes into the mouse germ line (*3*). The term "ES cell" was introduced to distinguish these embryo-derived pluripotent cells from teratocarcinoma-derived pluripotent embryonal carcinoma (EC) cells (*2*). Given the historical introduction of the term "ES cell" and the properties of mouse ES cells, we proposed that the essential characteristics of primate ES cells should include (i) derivation from the preimplantation or periimplantation embryo, (ii) prolonged undifferentiated proliferation, and (iii) stable developmental potential to form derivatives of all three embryonic germ layers even after prolonged culture (*4*). For ethical and practical reasons, in many primate species, including humans, the ability of ES cells to contribute to the germ line in chimeras is not a testable property. Nonhuman primate ES cell lines provide an accurate in vitro model for understanding the differentiation of human tissues (*4*, *5*). We now describe human cell lines that fulfill our proposed criteria to define primate ES cells.

Fresh or frozen cleavage stage human embryos, produced by in vitro fertilization (IVF) for clinical purposes, were donated by individuals after informed consent and after institutional review board approval. Embryos were cultured to the blastocyst stage, 14 inner cell masses were isolated, and five ES cell lines originating from five separate embryos were derived, essentially as described for nonhuman primate ES cells (*5*, *6*). The resulting cells had a high ratio of nucleus to cytoplasm, prominent nucleoli, and a colony morphology similar to that of rhesus monkey ES cells (Fig. 1). Three cell lines (H1, H13, and H14) had a normal XY karyotype, and two cell lines (H7 and H9) had a normal XX karyotype. Each of the cell lines was successfully cryopreserved and thawed. Four of the cell lines were cryopreserved after 5 to 6 months of continuous undifferentiated proliferation. The other cell line, H9, retained a normal XX karyotype after 6 months of culture and has now been passaged continuously for more than 8 months (32 passages). A period of replicative crisis was not observed for any of the cell lines.

The human ES cell lines expressed high levels of telomerase activity (Fig. 2). Telomerase is a ribonucleoprotein that adds telomere repeats to chromosome ends and is involved in maintaining telomere length, which plays an important role in replicative life-span (*7*, *8*). Telomerase expression is highly correlated with immortality in human cell lines, and reintroduction of telomerase activity into some diploid human somatic cell lines extends replicative life-span (*9*). Diploid human somatic cells do not express telomerase, have shortened telomeres with age, and enter replicative senescence after a finite proliferative life-span in tissue culture (*10*–*13*). In contrast, telomerase is present at high levels in germ line and embryonic tissues (*14*). The high level of telomerase activity expressed by the human ES cell lines therefore suggests that their replicative life-span will exceed that of somatic cells.

The human ES cell lines expressed cell surface markers that characterize undifferentiated nonhuman primate ES and human EC cells, including stage-specific embryonic antigen (SSEA)–3, SSEA-4, TRA-1-60, TRA-1-81, and alkaline phosphatase (Fig. 3) (*4*, *5*, *15*, *16*). The globo-series glycolipid GL7, which carries the SSEA-4 epitope, is formed by the addition of sialic acid to the globo-series glycolipid Gb5, which carries the SSEA-3 epitope (*17*, *18*). Thus, GL7 reacts with antibodies to both SSEA-3 and SSEA-4 (*17*, *18*). Staining intensity for SSEA-4 on the human ES cell lines was consistently strong, but staining intensity for SSEA-3 was weak and varied both within and among colonies (Fig. 3, D and C). Because GL7 carries both the SSEA-4 and SSEA-3 epitopes and because staining for SSEA-4 was consistently strong, the relatively weak staining for

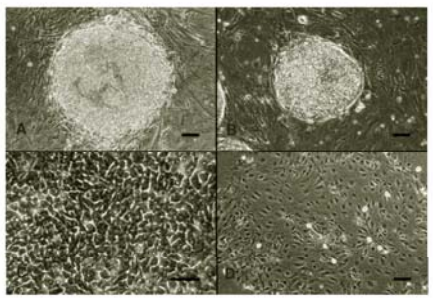

Fig. 1. Derivation of the H9 cell line. **(A)** Inner cell mass–derived cells attached to mouse embryonic fibroblast feeder layer after 8 days of culture, 24 hours before first dissociation. Scale bar, 100 μm. **(B)** H9 colony. Scale bar, 100 μm. **(C)** H9 cells. Scale bar, 50 μm. **(D)** Differentiated H9 cells, cultured for 5 days in the absence of mouse embryonic fibroblasts, but in the presence of human LIF (20 ng/ml; Sigma). Scale bar, 100 μm.

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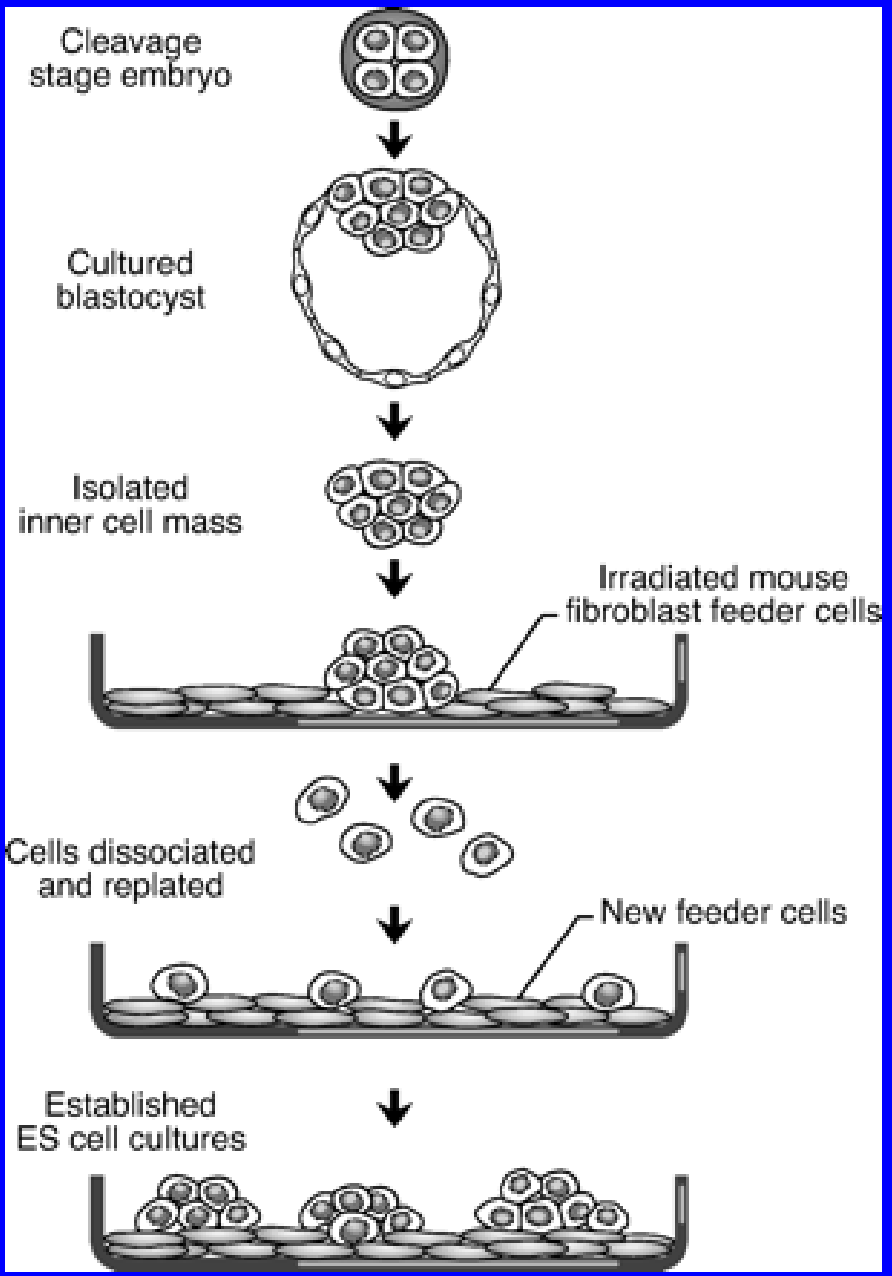
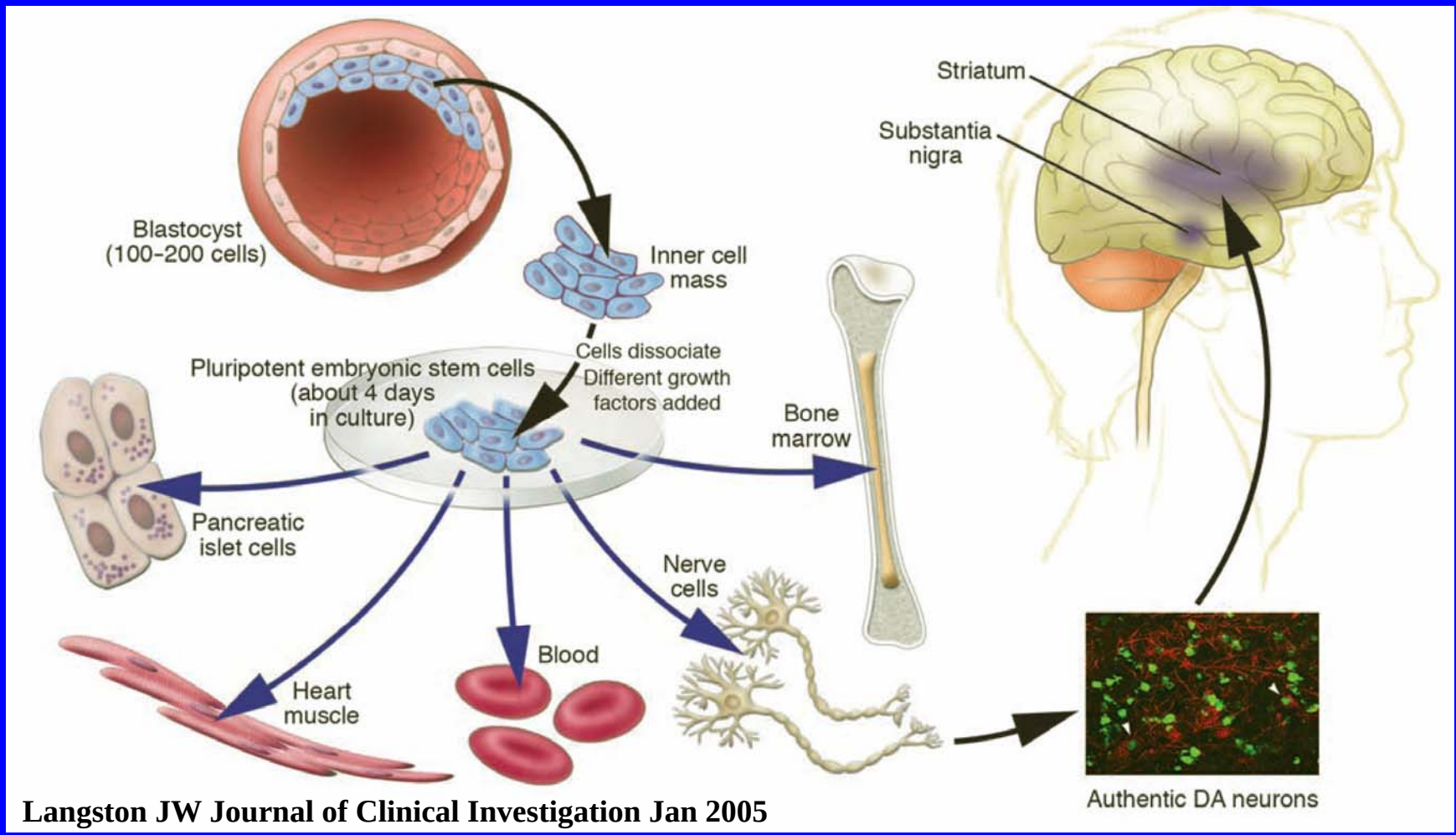
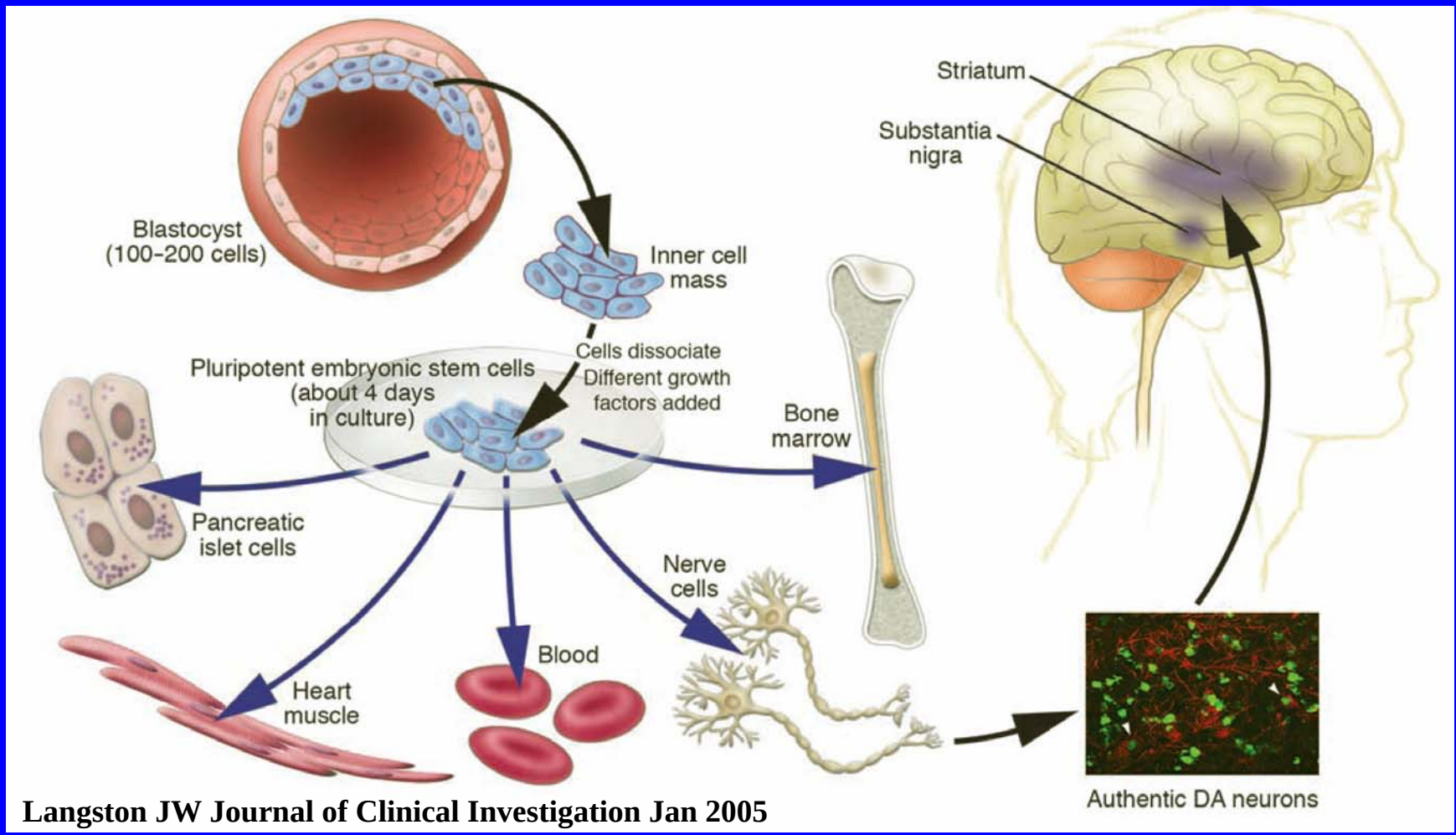


Potential applications of hESCs



Langston JW Journal of Clinical Investigation Jan 2005

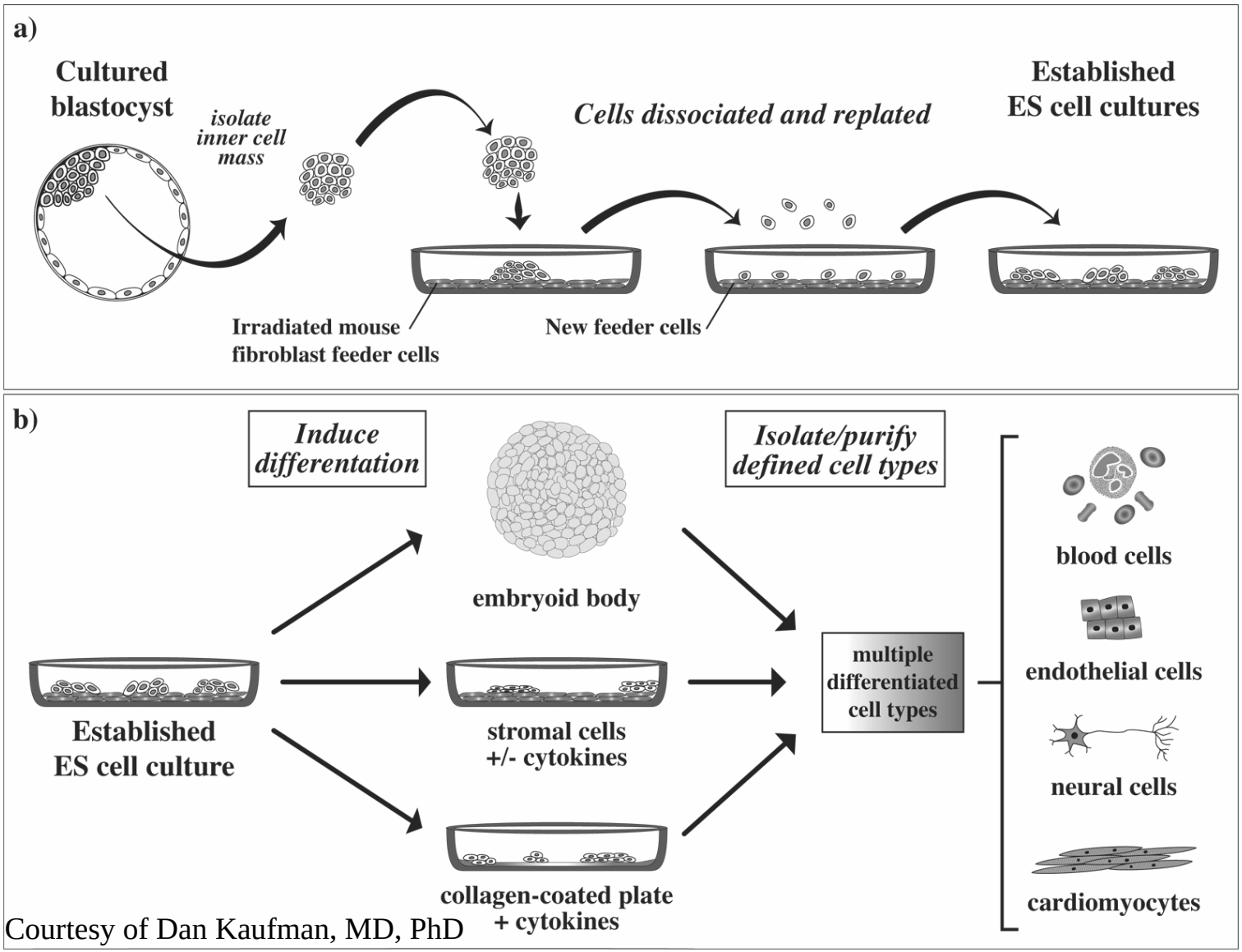
Potential applications of hESCs



Langston JW Journal of Clinical Investigation Jan 2005

- To Study Developmental Biology
- Drug Testing/Toxicology Screening

Protocols for directed differentiation of hESCs



Courtesy of Dan Kaufman, MD, PhD

Hematopoietic colony-forming cells derived from human embryonic stem cells

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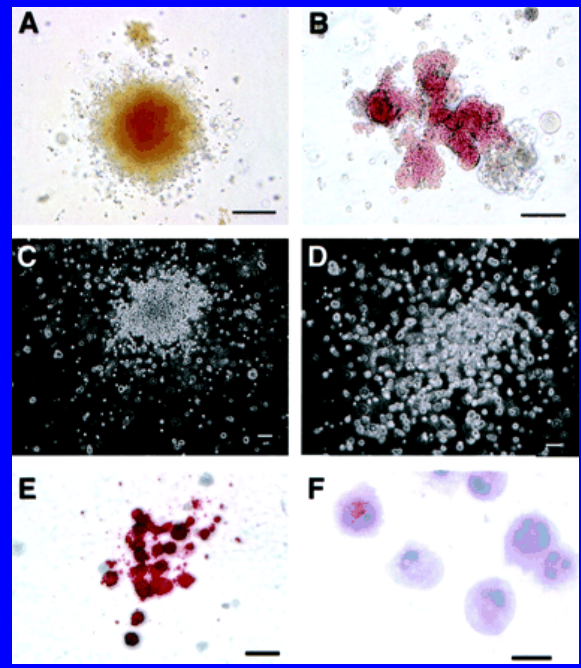
Communicated by Neal L. First, University of Wisconsin, Madison, WI, July 16, 2001 (received for review May 10, 2001)

Human embryonic stem (ES) cells are undifferentiated, pluripotent cells that can be maintained indefinitely in culture. Here we demonstrate that human ES cells differentiate to hematopoietic precursor cells when cocultured with the murine bone marrow cell line S17 or the yolk sac endothelial cell line C166. This hematopoietic differentiation requires fetal bovine serum, but no other exogenous cytokines. ES cell-derived hematopoietic precursor cells express the cell surface antigen CD34 and the hematopoietic transcription factors TAL-1, LMO-2, and GATA-2. When cultured on semisolid media with hematopoietic growth factors, these hematopoietic precursor cells form characteristic myeloid, erythroid, and megakaryocyte colonies. Selection for CD34⁺ cells derived from human ES cells enriches for hematopoietic colony-forming cells, similar to CD34 selection of primary hematopoietic tissue (bone marrow, umbilical cord blood). More terminally differentiated hematopoietic cells derived from human ES cells under these conditions also express normal surface antigens: glycophorin A on erythroid cells, CD15 on myeloid cells, and CD41 on megakaryocytes. The *in vitro* differentiation of human ES cells provides an opportunity to better understand human hematopoiesis and could lead to a novel source of cells for transfusion and transplantation therapies.

deficient mice (8–11). Moreover, some cells derived from non-hematopoietic tissue appear to have HSC potential (12, 13). The interrelationship between these varying sources and phenotypes of HSCs remains unclear.

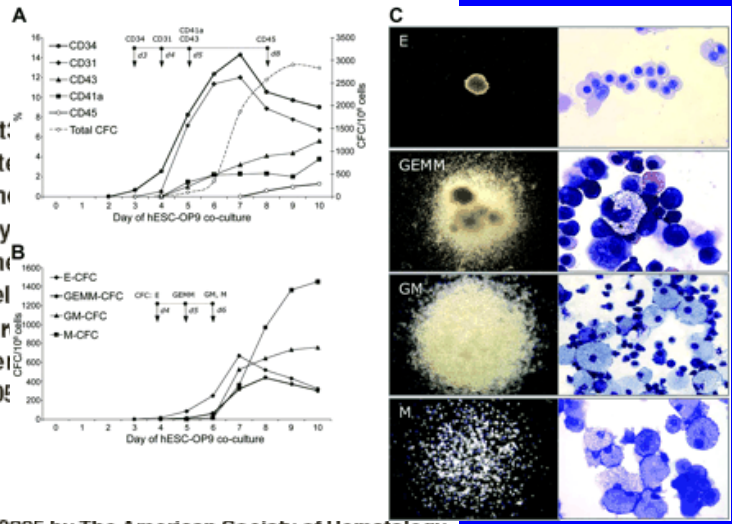
Human ES cells (14, 15) provide a unique, homogeneous, unlimited starting population of cells for studying human hematopoiesis. Human ES cells can be cultured for at least 300 population doubling times without observed senescence, while continuing to maintain normal karyotypes, telomere lengths, and pluripotency. Moreover, these cells can be cloned from a single cell without loss of pluripotency (16). Human ES cells give rise to differentiated cells and tissues from all three embryonic germ layers when allowed to form teratomas in immunodeficient mice or when induced to form embryoid bodies *in vitro* (14, 17). Mouse and human ES cells differ in morphology, population doubling time, and growth factor requirements. Undifferentiated mouse ES cells, for example, can be maintained as undifferentiated "feeder-independent" cells if growth factors such as leukemia inhibitory factor (LIF) or related cytokines are added to the media (1). If human ES cells are grown without feeder cells, but in the presence of LIF, they either differentiate or die (14, 15). Given the unexpected differences in the control of the



Human embryonic stem cell–derived CD34⁺ cells: efficient production in the coculture with OP9 stromal cells and analysis of lymphohematopoietic potential

Maxim A. Vodyanik, Jack A. Bork, James A. Thomson, and Igor I. Slukvin

Embryonic stem (ES) cells have the potential to serve as an alternative source of hematopoietic precursors for transplantation and for the study of hematopoietic cell development. Using coculture of human ES (hES) cells with OP9 bone marrow stromal cells, we were able to obtain up to 20% of CD34⁺ cells and isolate up to 10^7 CD34⁺ cells with more than 95% purity from a similar number of initially plated hES cells after 8 to 9 days of culture. The hES cell–derived CD34⁺ cells were highly enriched in colony-forming cells, cells expressing hematopoiesis-associated genes GATA-1, GATA-2, SCL/TAL1, and Flk-1, and retained clonogenic potential after in vitro expansion. CD34⁺ cells displayed the phenotype of primitive hematopoietic progenitors as defined by co-expression of CD90, CD117, and CD164, along with a lack of CD38 expression and contained aldehyde dehydrogenase–positive cells as well as cells with verapamil-sensitive ability to efflux rhodamine 123. When cultured on MS-5 stromal cells in the presence of stem cell factor,



Hypothesis:
Wherever there is Blood, there should be Mesenchymal Cells!

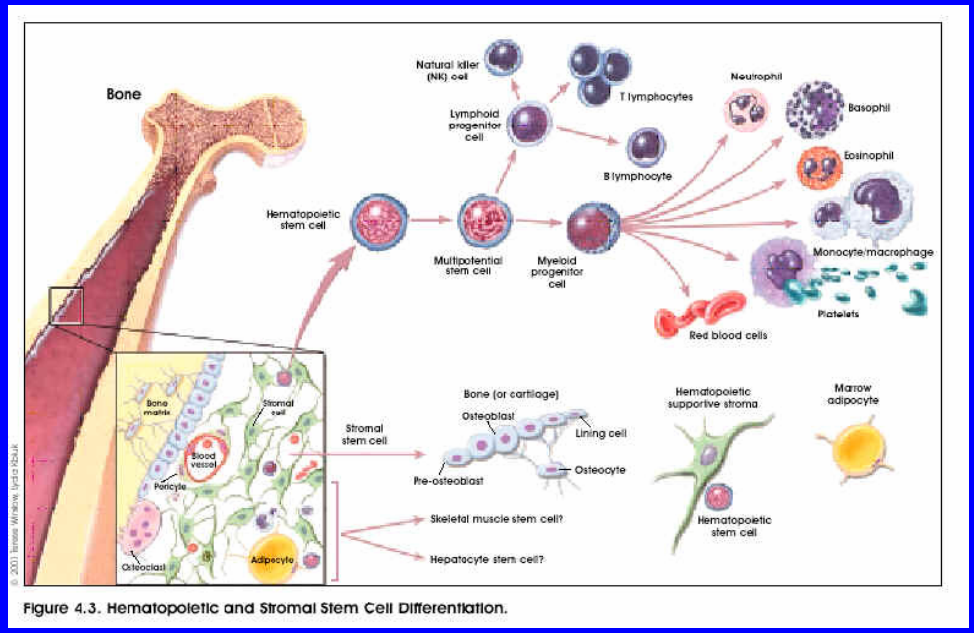
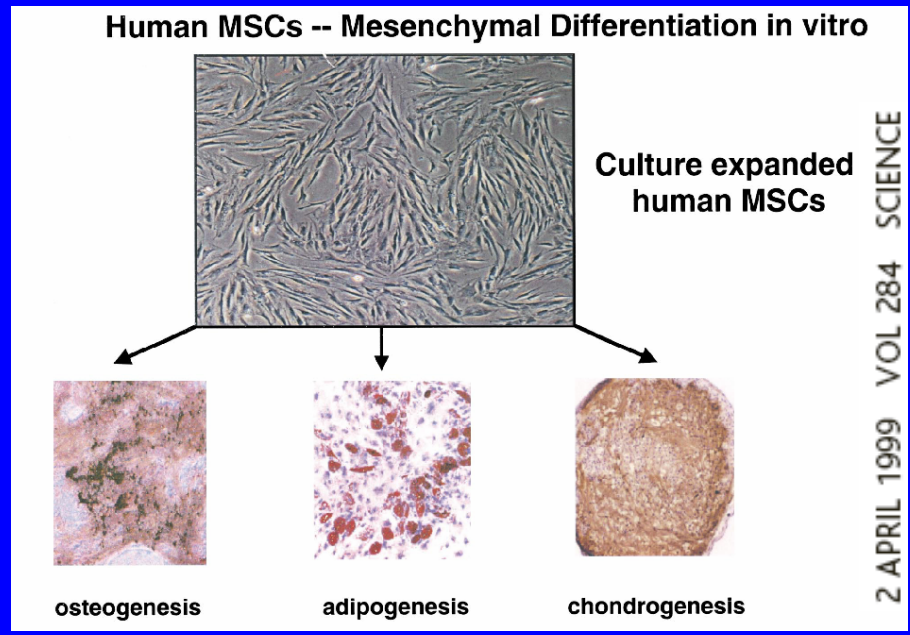


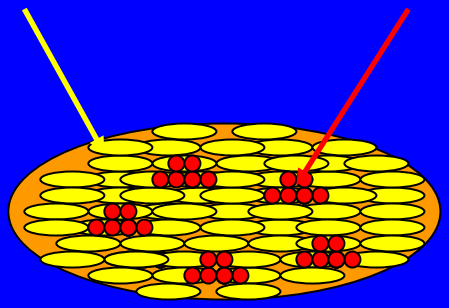
Figure 4.3. Hematopoietic and Stromal Stem Cell Differentiation.



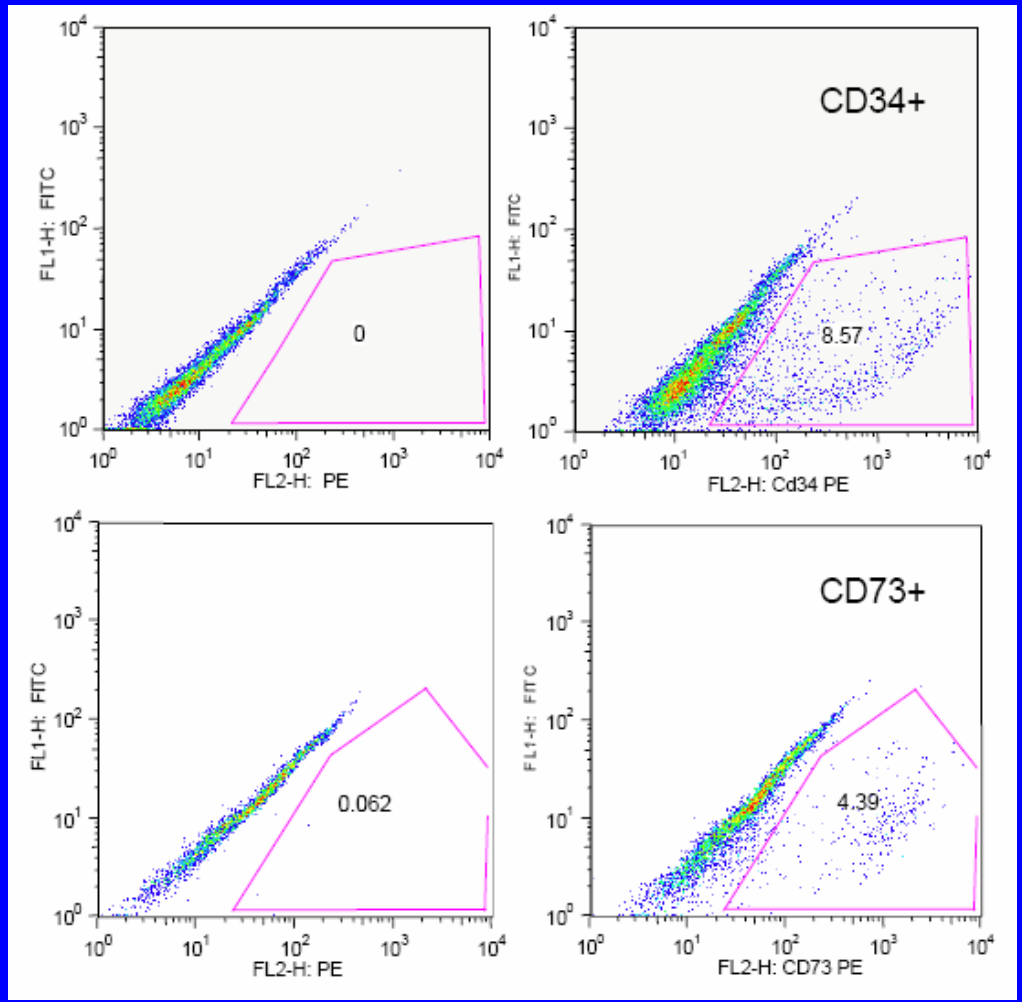
Simultaneous generation of CD34+ and CD73+ cells from hESCs co-cultured with OP9 murine bone marrow stromal cells

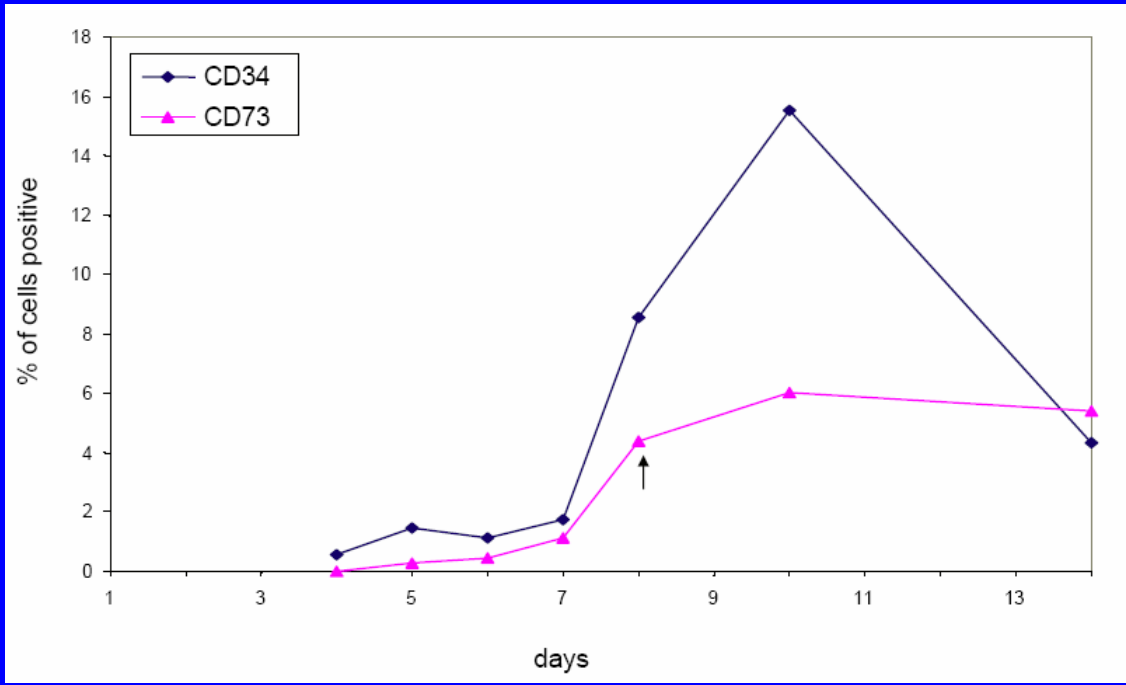
Mouse OP9 cells

hESCs

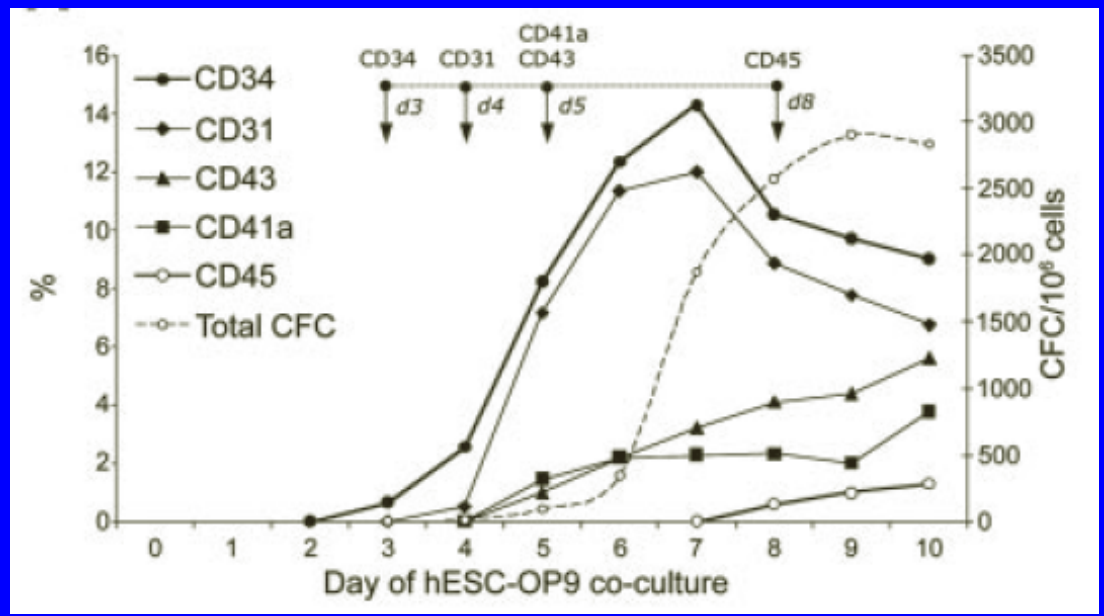


Day 8 of co-culture

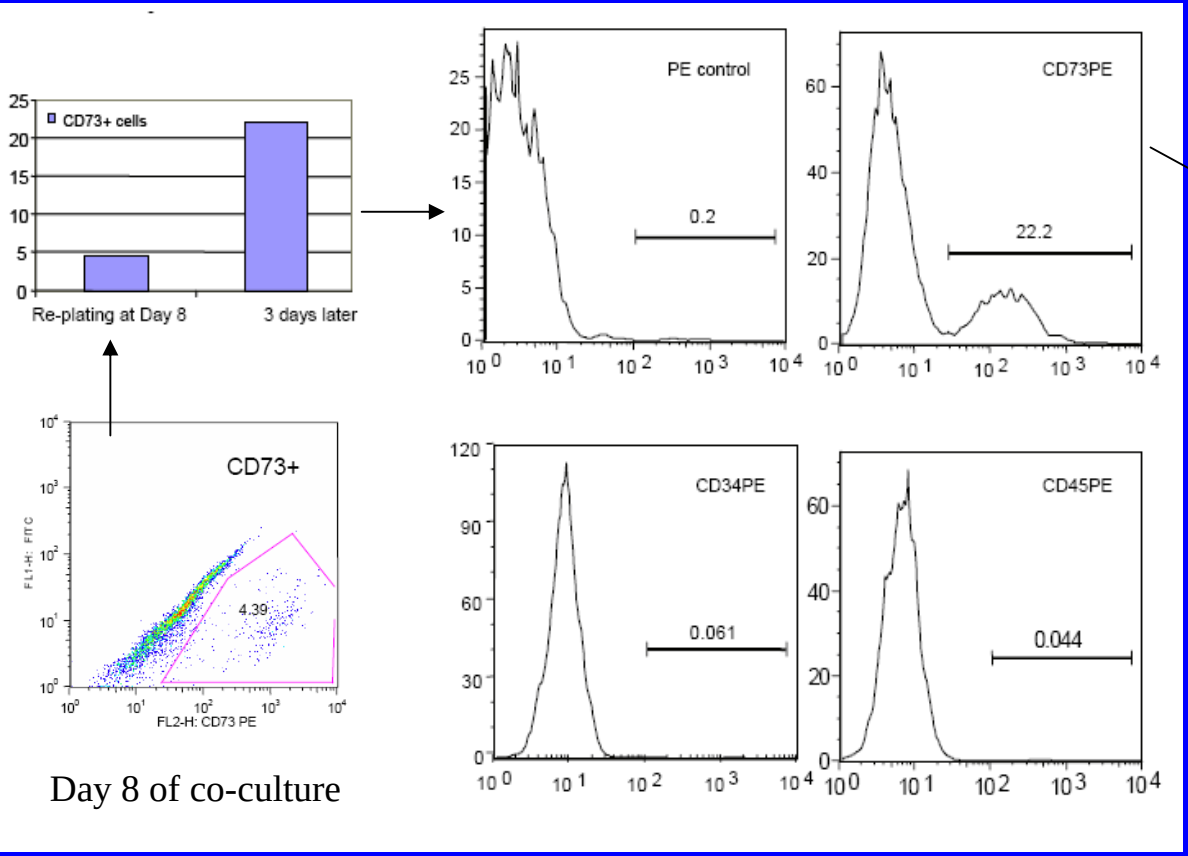




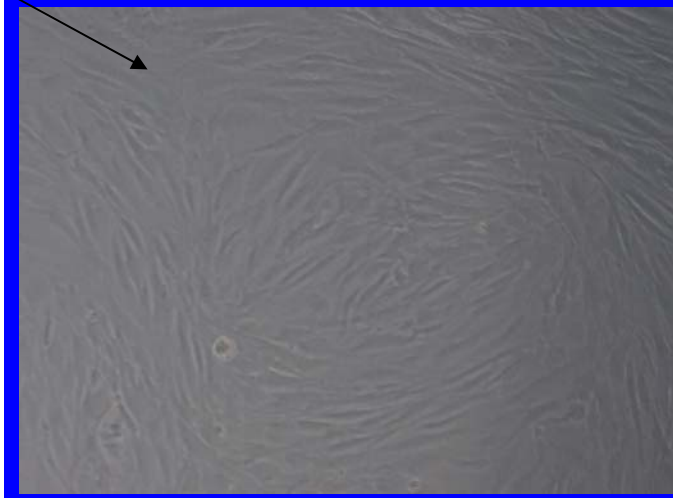
Kinetics of generation of CD34+ and CD73+ Cells in the first 2 weeks of co-culture



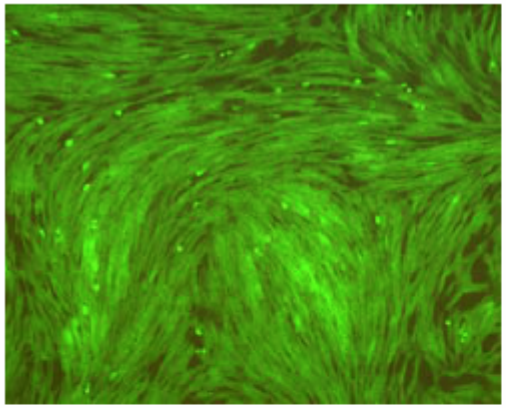
Generation of a pure population of CD73+ mesenchymal cells from hESCs co-cultured with OP9 cells

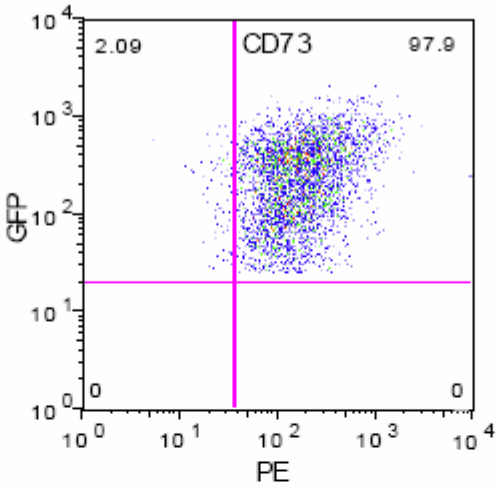


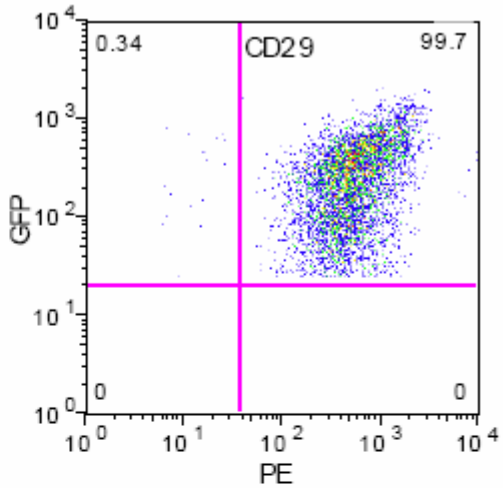
Day 8 of co-culture

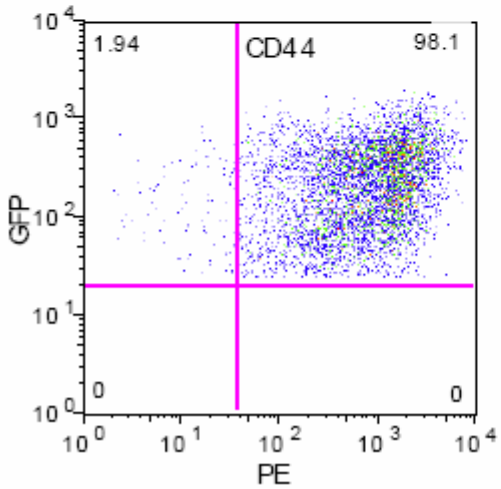


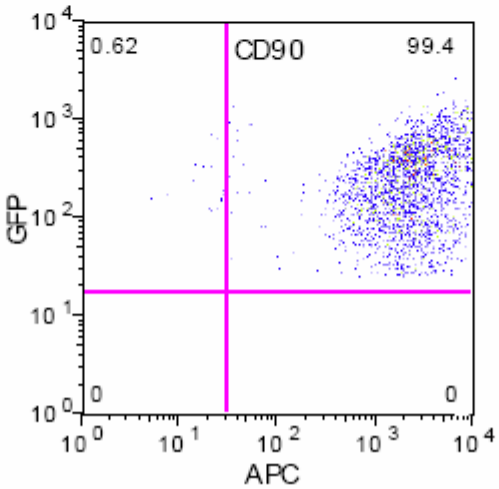
Generation of GFP+/MSCs from GFP+/hESCs co-cultured with OP9

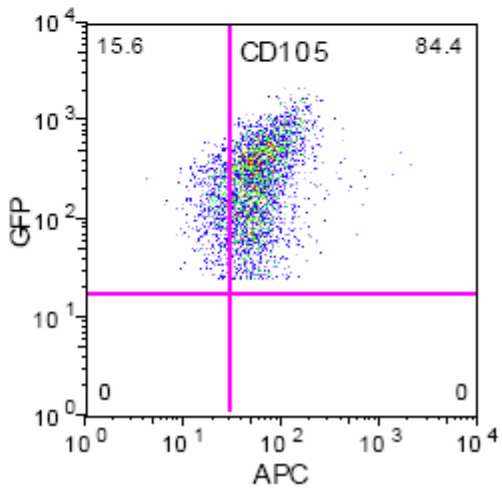




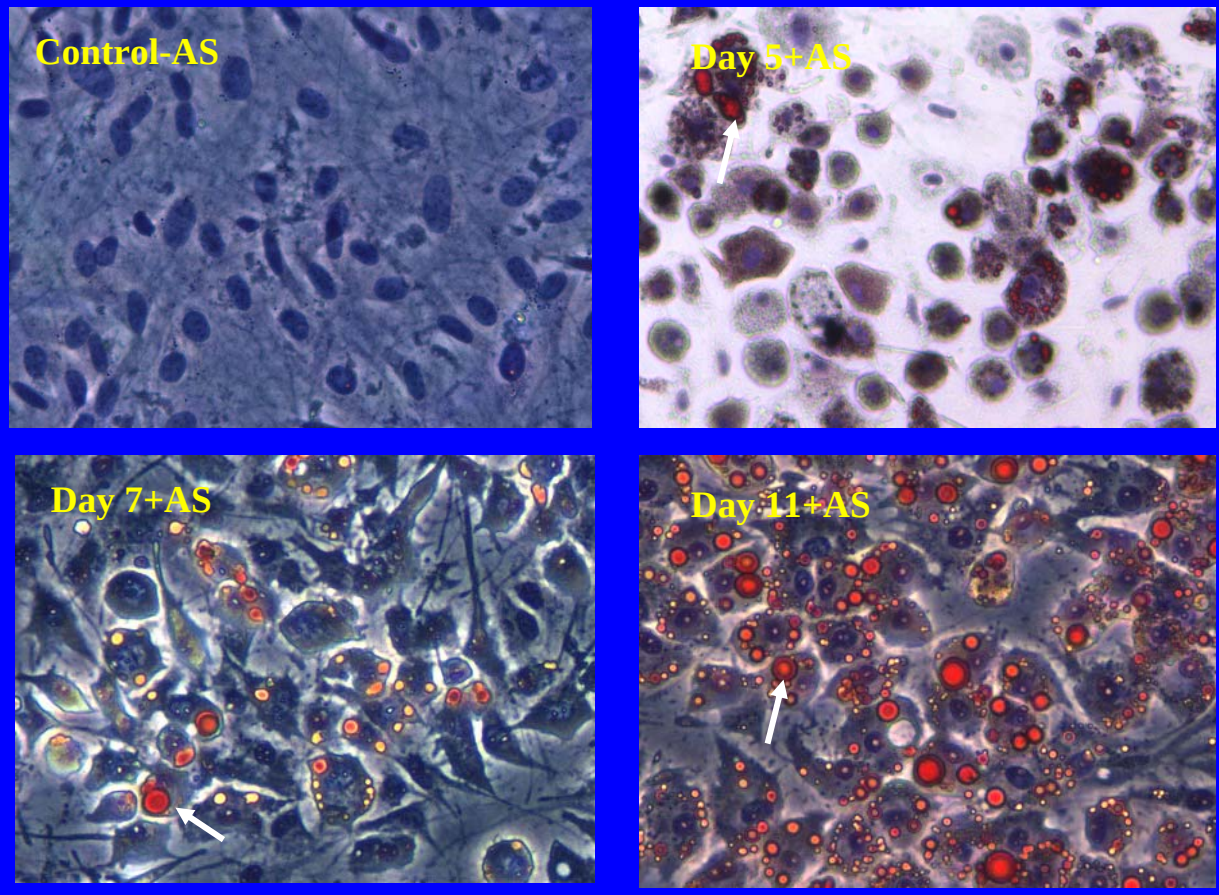






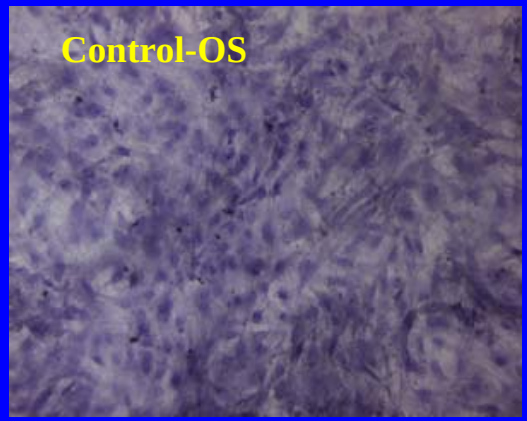


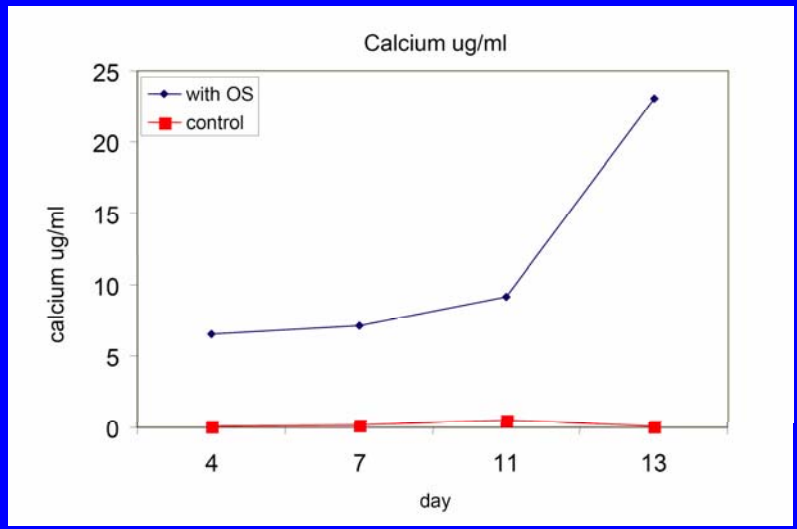
Adipogenic potential of ESC-derived CD73+ MSCs



Adipogenic Supplement =Isobutylxanthine+Insulin

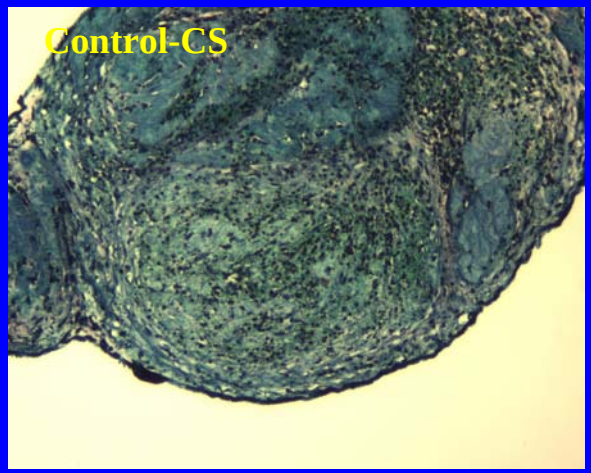
Osteogenic potential of ESC-derived CD73+ MSCs

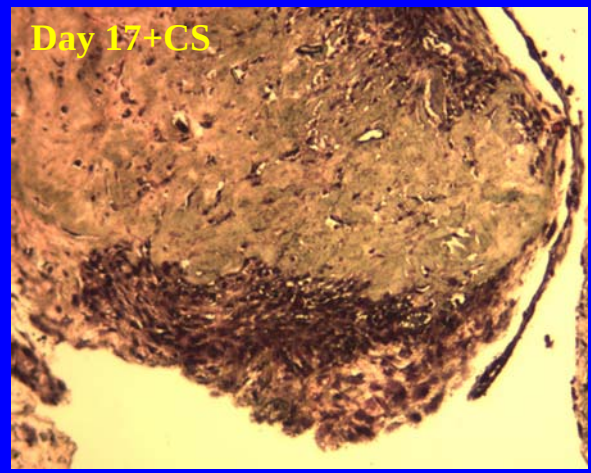




Osteogenic supplement=Beta Glycerol+Ascorbic acid

Chondrogenic potential of ESC-derived CD73+ MSCs





Chondrogenic supplement=TGF Beta

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Derivation of Multipotent Mesenchymal Precursors from Human Embryonic Stem Cells

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Competing Interests: The authors have declared that no competing interests exist.

Author Contributions: TB and LS designed the study. TB and LMW performed the experiments. TB, LMW, NDS, and LS analyzed the data. TB, NDS, and LS contributed to writing the paper.

Academic Editor: Sally Temple, Albany Medical College, United States of America

Citation: Barberi T, Willis LM, Socci ND, Studer L (2005) Derivation of multipotent mesenchymal precursors from human embryonic stem cells. PLoS Med 2(6): e161.

Received: October 13, 2004
Accepted: April 15, 2005
Published: June 28, 2005

DOI: 10.1371/journal.pmed.0020161

Abbreviations: ES, embryonic stem; FACS, flow-activated cell sorting; FBS, fetal bovine serum; hESC, human embryonic stem cell; hESMPC, human embryonic stem cell–derived mesenchymal precursor cell; MSC, mesenchymal stem cell

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ABSTRACT

Background

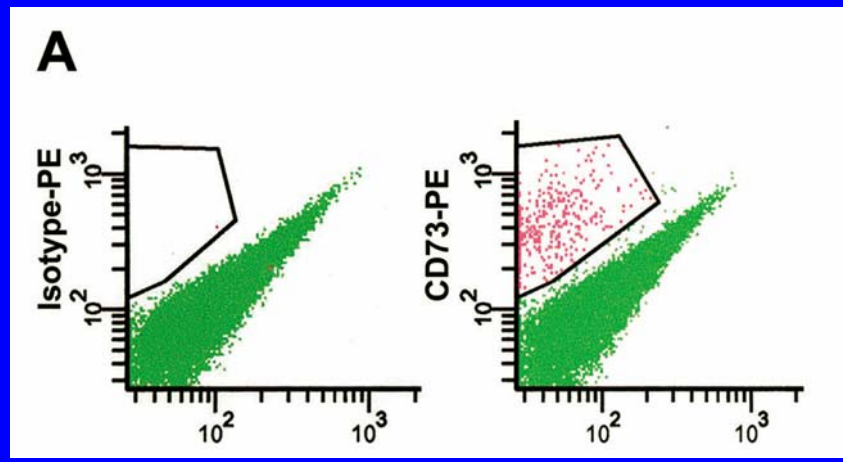
Human embryonic stem cells provide access to the earliest stages of human development and may serve as a source of specialized cells for regenerative medicine. Thus, it becomes crucial to develop protocols for the directed differentiation of embryonic stem cells into tissue-restricted precursors.

Methods and Findings

Here, we present culture conditions for the derivation of unlimited numbers of pure mesenchymal precursors from human embryonic stem cells and demonstrate multilineage differentiation into fat, cartilage, bone, and skeletal muscle cells.

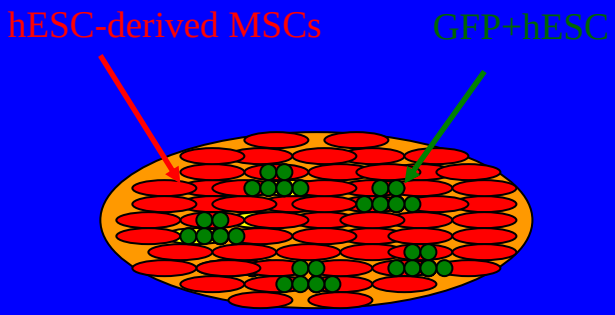
Conclusion

Our findings will help to elucidate the mechanism of mesoderm specification during embryonic stem cell differentiation and provide a platform to efficiently generate specialized human mesenchymal cell types for future clinical applications.

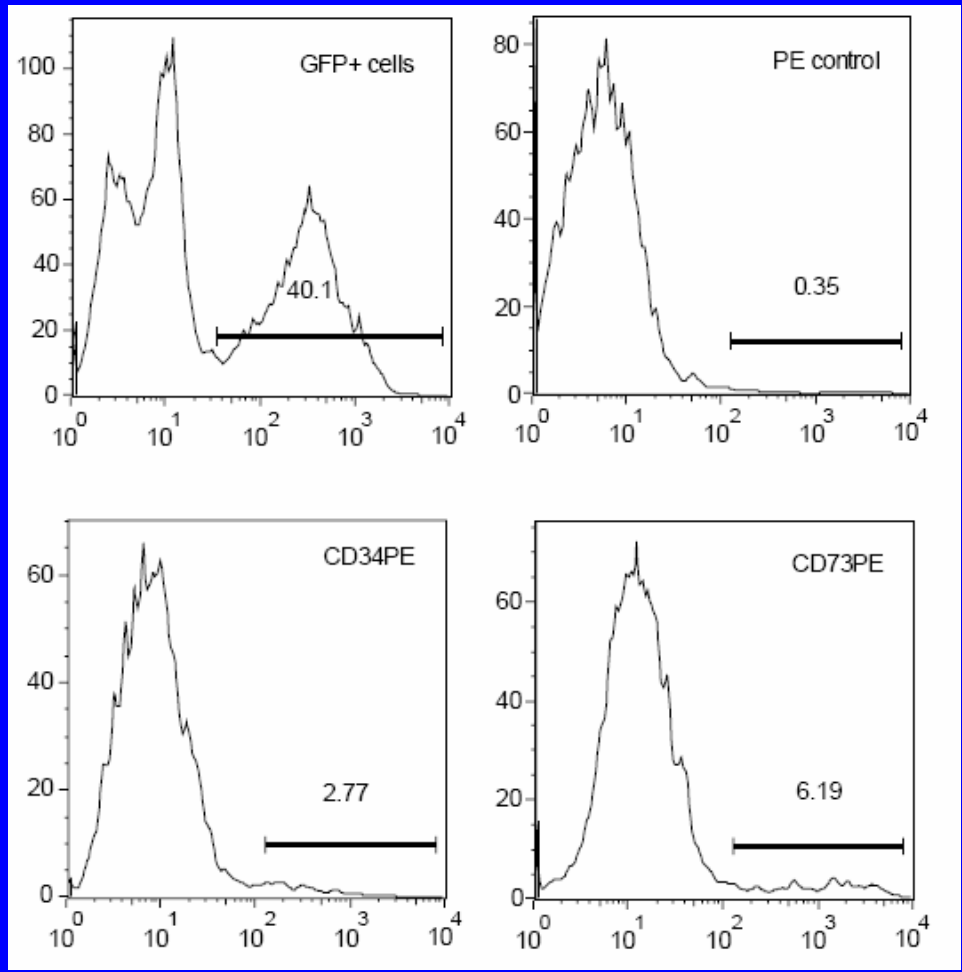


After 40 days of co-culturing hESCs with OP9 cells

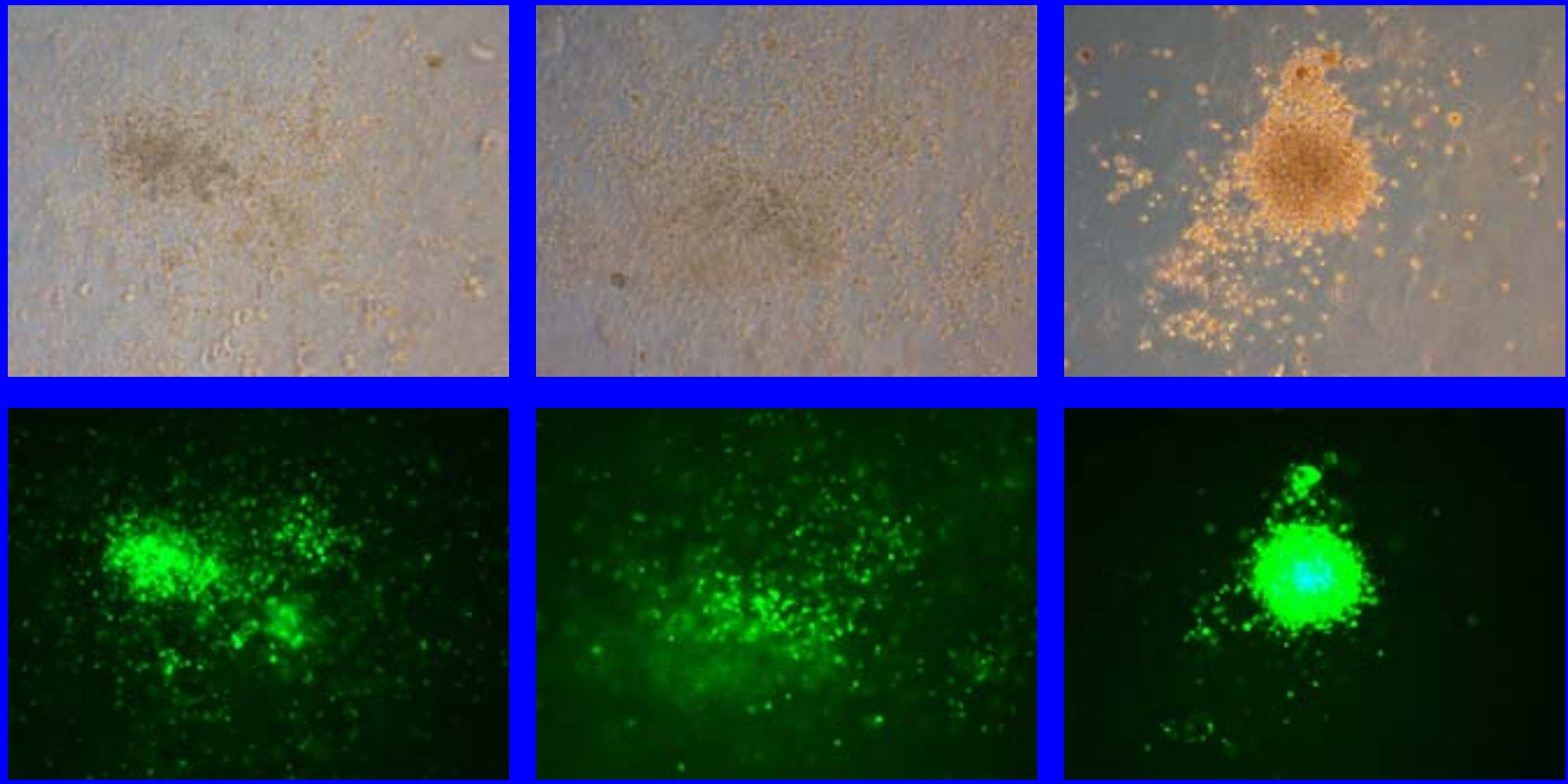
Are hESC-derived MSCs capable of supporting generation of CD34+ & CD73+ cells from hESCs?

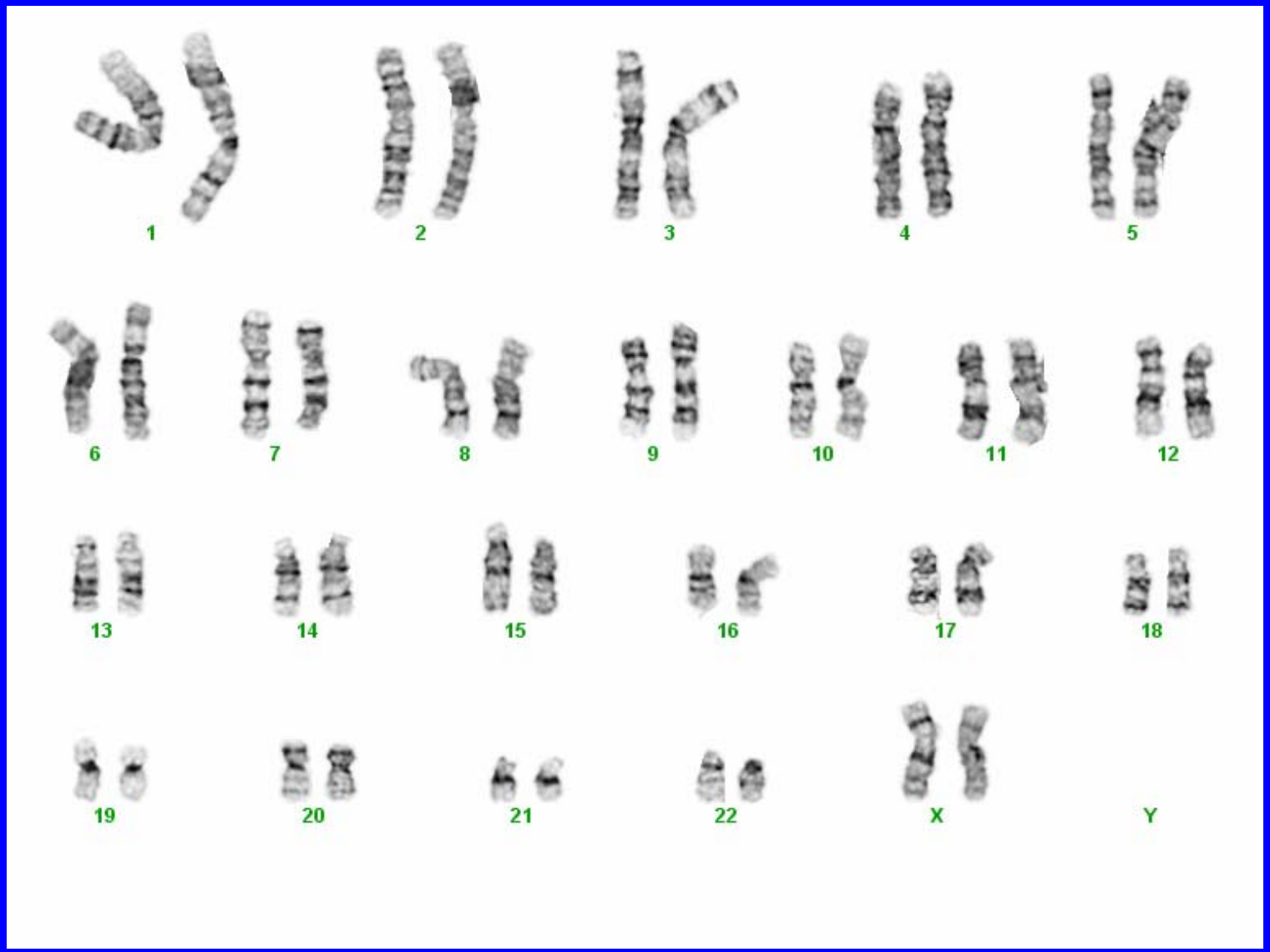


GFP+/hESCs co-cultured with hESC-derived MSCs



Hematopoietic colonies derived from GFP+/hESCs co-cultured with hESC-derived CD73+/MSCs (not CD34+ selected)





Karyotypic analysis of hESC-derived MSCs

Potential Clinical Applications of Mesenchymal Stem Cells

Rapid Hematopoietic Recovery After Coinfusion of Autologous-Blood Stem Cells and Culture-Expanded Marrow Mesenchymal Stem Cells in Advanced Breast Cancer Patients Receiving High-Dose Chemotherapy

By Omer N. Koç, Stanton L. Gerson, Brenda W. Cooper, Stephanie M. Dyhouse, Stephen E. Haynesworth, Arnold I. Caplan, and Hillard M. Lazarus

Purpose: **Multipotential mesenchymal stem cells (MSCs) are found in human bone marrow and are shown to secrete hematopoietic cytokines and support hematopoietic progenitors in vitro. We hypothesized that infusion of autologous MSCs after myeloablative therapy would facilitate engraftment by hematopoietic stem cells, and we investigated the feasibility, safety, and hematopoietic effects of culture-expanded MSCs in breast cancer patients receiving autologous peripheral-blood progenitor-cell (PBPC) infusion.**

Patients and Methods: **We developed an efficient method of isolating and culture-expanding a homogenous population of MSCs from a small marrow-aspirate sample obtained from 32 breast cancer patients. Twenty-eight patients were given high-dose chemotherapy and autologous PBPCs plus culture-expanded MSC infusion and daily granulocyte colony-stimulating factor.**

Results: **Human MSCs were successfully isolated from a mean ± SD of 23.4 ± 5.9 mL of bone marrow aspirate from all patients. Expansion cultures generated greater than 1×10^6 MSCs/kg for all patients over 20 to 50 days with a mean potential of 5.6 to 36.3 × 10^6 MSCs/kg after two to six passages, respectively. Twenty-eight patients were infused with 1 to 2.2 × 10^6 expanded autologous MSCs/kg intravenously over 15 minutes. There were no toxicities related to the infusion of MSCs. Clonogenic MSCs were detected in venous blood up to 1 hour after infusion in 13 of 21 patients (62%). Median time to achieve a neutrophil count greater than 500/μL and platelet count ≥ 20,000/μL untransfused was 8 days (range, 6 to 11 days) and 8.5 days (range, 4 to 19 days), respectively.**

Conclusion: **This report is the first describing infusion of autologous MSCs with therapeutic intent. We found that autologous MSC infusion at the time of PBPC transplantation is feasible and safe. The observed rapid hematopoietic recovery suggests that MSC infusion after myeloablative therapy may have a positive impact on hematopoiesis and should be tested in randomized trials.**

J Clin Oncol 18:307-316.

Brief report

Human embryonic stem cell–derived hematopoietic cells are capable of engrafting primary as well as secondary fetal sheep recipients

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A. Daisy Narayan, Jessica L. Chase, Rachel L. Lewis, Xinghui Tian, Dan S. Kaufman, James A. Thomson, and Esmail D. Zanjani

The human/sheep xenograft model has proven valuable in assessing the in vivo hematopoietic activity of stem cells from a variety of fetal and postnatal human sources. $CD34^+$/$lineage^-$ or $CD34^+$/$CD38^-$ cells isolated from human embryonic stem cells (hESCs) differentiated on S17 feeder layer were transplanted by intraperitoneal injections into fetal sheep. Chimerism in primary transplants was established with polymerase chain reaction (PCR) and flow cytometry of bone marrow and peripheral blood samples. Whole bone marrow cells harvested from a primary recipient were transplanted into a secondary recipient. Chimerism was established as described before. This animal was stimulated with human GM-CSF, and an increase in human hematopoietic activity was noted by flow cytometry. Bone marrow aspirations cultured in methylcellulose generated colonies identified by PCR to be of human origin. We therefore conclude that hESCs are capable of generating hematopoietic cells that engraft primary recipients. These cells also fulfill the criteria for long-term engrafting hematopoietic stem cells as demonstrated by engraftment and differentiation in the secondary recipient. (Blood. 2006;107:2180-2183)

Autologous Cultured Chondrocytes: Adverse Events Reported to the United States Food and Drug Administration

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CLINICAL OBSERVATIONS, INTERVENTIONS, AND THERAPEUTIC TRIALS

BLOOD, 1 MARCH 2001 • VOLUME 97, NUMBER 5

Clinical responses to bone marrow transplantation in children with severe osteogenesis imperfecta

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Preclinical models have shown that transplantation of marrow mesenchymal cells has the potential to correct inherited disorders of bone, cartilage, and muscle. The report describes clinical responses of the first children to undergo allogeneic bone marrow transplantation (BMT) for severe osteogenesis imperfecta (OI), a genetic disorder characterized by defective type I collagen, osteopenia, bone fragility, severe bony deformities, and growth retardation. Five children with severe OI were enrolled in a study of BMT from human leukocyte antigen (HLA)–compatible sibling donors. Linear growth, bone mineralization, and fracture rate were taken as measures of treatment response. The 3 children with documented donor osteoblast engraftment had a median 7.5-cm increase in body length (range, 6.5-8.0 cm) 6 months after transplantation compared with 1.25 cm (range, 1.0-1.5 cm) for age-matched control patients. These patients gained 21.0 to 65.3 g total body bone mineral content by 3 months after treatment or 45% to 77% of their baseline values. With extended follow-up, the patients' growth rates either slowed or reached a plateau phase. Bone mineral content continued to increase at a rate similar to that for weight-matched healthy children, even as growth rates declined. These results suggest that BMT from HLA-compatible donors may benefit children with severe OI. Further studies are needed to determine the full potential of this strategy. (Blood. 2001;97:1227-1231)

Human mesenchymal stem cells modulate allogeneic immune cell responses

Sudeepta Aggarwal and Mark F. Pittenger

Mesenchymal stem cells (MSCs) are multipotent cells found in several adult tissues. Transplanted allogeneic MSCs can be detected in recipients at extended time points, indicating a lack of immune recognition and clearance. As well, a role for bone marrow–derived MSCs in reducing the incidence and severity of graft-versus-host disease (GVHD) during allogeneic transplantation has recently been reported; however, the mechanisms remain to be investigated. We examined the immunomodulatory functions of human MSCs (hMSCs) by coculturing them with purified subpopulations of immune cells and report here that hMSCs altered the cytokine secretion profile of dendritic cells (DCs), naive and effector T cells (T helper 1 [T_H1] and T_H2), and natural killer (NK) cells to induce a more anti-inflammatory or tolerant phenotype. Specifically, the hMSCs caused mature DCs type 1 (DC1) to decrease tumor necrosis factor α (TNF-α) secretion and mature DC2 to increase interleukin-10 (IL-10) secretion; hMSCs caused T_H1 cells to decrease interferon γ (IFN-γ) and caused the T_H2 cells to increase secretion of IL-4; hMSCs caused an increase in the proportion of regulatory T cells (T_{Regs}) present; and hMSCs decreased secretion of IFN-γ from the NK cells. Mechanistically, the hMSCs produced elevated prostaglandin E2 (PGE_2) in co-cultures, and inhibitors of PGE_2 production mitigated hMSC-mediated immune modulation. These data offer insight into the interactions between allogeneic MSCs and immune cells and provide mechanisms likely involved with the in vivo MSC-mediated induction of tolerance that could be therapeutic for reduction of GVHD, rejection, and modulation of inflammation. (Blood. 2005;105:1815-1822)

Introduction

The isolation of stem cell populations has burgeoned in the last 10 years, opening many new opportunities to evaluate the stem cells and their use in tissue regeneration. Hematopoietic stem cells (HSCs) were identified after a long search for cells that would allow survival following radiation exposure. During this period, several studies demonstrated that bone marrow would form new bone when transplanted to an ectopic site.[1] The isolation and culture of cells from bone marrow that could form this ectopic bone were first demonstrated by Friedenstein et al[2] using the guinea pig as a model. Later, several groups published similar data for rat and rabbit cells harvested from bone marrow and other tissues.[2-4] Similar to the hematopoietic stem cell and its lineages, the concept emerged that there may be a mesenchymal stem cell (MSC), a single cell capable of forming bone, cartilage, and other mesenchymal tissues.[3,4] Haynesworth et al[5] developed a reliable in vivo bone-forming assay and were able to isolate and culture human MSCs in therapeutic quantities.

Subsequently, in vitro experiments demonstrated that clonal human MSCs are able to differentiate into various lineages including osteoblasts, chondrocytes, and adipocytes.[6,7] In vitro and in vivo studies have also indicated the capability of MSCs to differentiate into muscle,[8] neural precursors,[9,10] cardiomyocytes,[11-13] and possibly other cell types.[14,15] In addition, MSCs have been shown to provide cytokine and growth factor support for expansion of hematopoietic and embryonic stem cells.[16-19] Numerous studies with a variety of animal models have shown that MSCs may be useful in the repair or regeneration of myocardial tissues,[13,20,21] damaged bone,[22-25] tendon,[26] cartilage,[27] and meniscus.[28] Perhaps one of the most remarkable and least understood findings is the ability of MSCs to migrate to sites of tissue injury.[13,29-31] Several clinical studies using autologous whole bone marrow, presumably containing MSCs, HSCs, and/or endothelial progenitor cells have also been reported for patients with myocardial infarcts.[32-34] Importantly, encouraging results have been reported for ex vivo–cultured MSCs in early clinical use including engraftment of autologous[35,36] or allogeneic[37] (also Lazarus et al, manuscript submitted, August 2004) bone marrow transplants, allogeneic MSCs for the collagen I genetic disease osteogenesis imperfecta,[38] and recently for the treatment of graft-versus-host disease (GVHD).[39]

Human MSCs (hMSCs) can be isolated from several, perhaps most, tissues, although bone marrow is most often used. Human MSCs express intermediate levels of human leukocyte antigen (HLA) major histocompatibility complex (MHC) class I, and they can be induced to express MHC class II antigen[40-43] and Fas ligand by interferon γ (IFN-γ) treatment. MSCs do not express costimulatory molecules B7-1, B7-2, CD40, and CD40 ligand and probably, therefore, do not activate alloreactive T cells.[41,43,44] In addition, MSCs differentiated into various mesenchymal lineages do not appear to alter their interaction with T cells.[45,46] MSCs isolated from humans and other mammalian species including baboon, canine, caprine, and rodents do not elicit a proliferative response

From Osiris Therapeutics, Baltimore, MD.

Submitted April 26, 2004; accepted September 26, 2004. Prepublished online as *Blood* First Edition Paper, October 19, 2004; DOI 10.1182/blood-2004-04-1559.

Supported in part by Defense Advanced Research Projects Agency (DARPA) grant no. N66001-02-C-8068.

S.A. and M.F.P. are currently employed at Osiris Therapeutics, Inc, which is developing cellular therapeutics based on human mesenchymal stem cells.

The online version of the article contains a data supplement.

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Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells

Katarina Le Blanc, Ida Rasmusson, Berit Sundberg, Cecilia Götherström, Moustapha Hassan, Mehmet Uzunel, Olle Ringdén

Adult bone-marrow-derived mesenchymal stem cells are immunosuppressive and prolong the rejection of mismatched skin grafts in animals. We transplanted haploidentical mesenchymal stem cells in a patient with severe treatment-resistant grade IV acute graft-versus-host disease of the gut and liver. Clinical response was striking. The patient is now well after 1 year. We postulate that mesenchymal stem cells have a potent immunosuppressive effect in vivo.

Lancet 2004; **363:** 1439–41
See Commentary page 1411

Severe acute graft-versus-host disease (GVHD) after allogeneic stem-cell transplantation is associated with high mortality. Bone marrow contains pluripotent mesenchymal stem cells that form bone, cartilage, adipose tissue, and muscle. These stem cells are not immunogenic and escape recognition by alloreactive T cells and natural killer cells. Mesenchymal stem cells given intravenously have been well tolerated.[1] Furthermore, they are immunosuppressive and inhibit the proliferation of alloreactive T cells. Preliminary reports of co-transplantation of mesenchymal stem cells and haemopoietic stem cells from HLA-identical siblings show a reduction in acute and chronic GVHD.[2] We describe our experience of transplanting haploidentical mesenchymal stem cells to treat severe GVHD after allogeneic stem-cell transplantation.

A 9-year-old boy with acute lymphoblastic leukaemia in third remission received a transplant of blood stem cells from an HLA-A, HLA-B, HLA-DRβ1 identical, unrelated, female donor after conditioning with cyclophosphamide (120 mg/kg) and fractionated total body irradiation (3 Gy for 4 days). Immunosuppression included thymoglobulin (6 mg/kg) during the conditioning, followed by ciclosporin combined with four doses of methotrexate. On day 11 after allogeneic stem-cell transplantation, the patient developed a maculopapular rash of the thorax and back that progressed despite treatment with prednisolone (2 mg/kg daily). By day 22, the patient developed diarrhoea (>1000 mL per day) and abdominal pain requiring morphine. He stopped eating on day 24. Bilirubin and alanine aminotransferase concentrations rose (figure 1). Psoralen and ultraviolet-A light (PUVA) treatment (two to three times per week) for 3 weeks was followed by extracorporeal PUVA (one to four times per week) for 6 weeks. Infliximab (10 mg/kg) and daclizumab (1 mg/kg) for 4 weeks were ineffective (figure 1). Methylprednisolone (250–500 mg per day) for a total of 22 days, mycophenolate mofetil, and methotrexate were also tried. By day 70, the patient had developed grade IV acute GVHD, including diarrhoea up to 20 times daily, and a bilirubin concentration of 250 mmol/L. He was treated for repeated bacterial, viral, and invasive fungal infections.

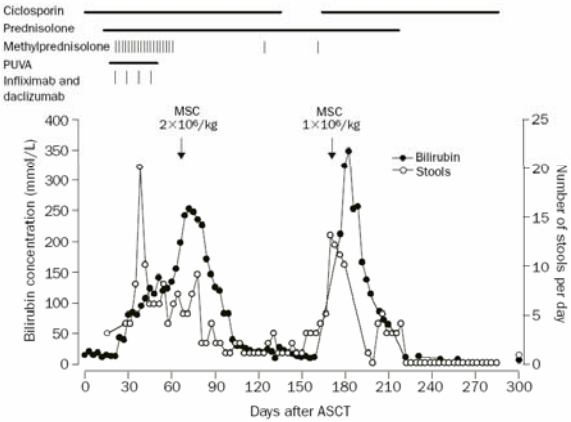

Figure 1: **Clinical course and immunosuppression of the patient**
↓=mesenchymal stem-cell transplantation. ASCT=allogeneic stem-cell transplantation. MSC=mesenchymal stem cells.

We chose the mother as donor, because she was readily available and because MHC-compatibility is not necessary for mesenchymal stem-cell immunosuppression.[3] After ethics-committee approval and informed consent, mesenchymal stem cells were isolated as previously described.[3] Briefly, we isolated mononuclear cells from a bone marrow aspirate by Percoll gradient centrifugation (Sigma, St Louis, MO, USA). The cells were plated at a density of 3×10^7 cells per 175 cm^2 in polystyrene flasks in low glucose Dulbecco's modified Eagle's medium (Life Technologies, Gaithersburg, MD, USA), supplemented with 10% fetal bovine serum (National Veterinary Institute, Uppsala, Sweden) and 1% antibiotic-antimycotic solution (Life Technologies). We selected the serum lot on the basis of optimum mesenchymal stem-cell growth with maximum retention of osteogenic, chondrogenic, and adipogenic differentiation. When the cultures were near confluence, the cells

Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction

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Communicated by Victor A. McKusick, The Johns Hopkins University School of Medicine, Baltimore, MD, June 2, 2005 (received for review April 13, 2005)

Although clinical trials of autologous whole bone marrow for cardiac repair demonstrate promising results, many practical and mechanistic issues regarding this therapy remain highly controversial. Here, we report the results of a randomized study of bone-marrow-derived mesenchymal stem cells, administered to pigs, which offer several new insights regarding cellular cardiomyoplasty. First, cells were safely injected by using a percutaneous-injection catheter 3 d after myocardial infarction. Second, cellular transplantation resulted in long-term engraftment, profound reduction in scar formation, and near-normalization of cardiac function. Third, transplanted cells were preprepared from an allogeneic donor and were not rejected, a major practical advance for widespread application of this therapy. Together, these findings demonstrate that the direct injection of cellular grafts into damaged myocardium is safe and effective in the periinfarct period. The direct delivery of cells to necrotic myocardium offers a valuable alternative to intracoronary cell injections, and the use of allogeneic mesenchymal stem cells provides a valuable strategy for cardiac regenerative therapy that avoids the need for preparing autologous cells from the recipient.

There is growing enthusiasm for the application of bone-marrow-derived cell-based therapies to repair or regenerate damaged myocardium. Small clinical trials conducted in the perimyocardial infarction (MI) period (1–5) with intracoronary infusion of autologous whole bone marrow preparations have suggested moderate improvements in cardiac function (1–3). However, recent experimental studies questioning the engraftment of hematopoietic stem cells (6, 7) and clinical findings that bone marrow cells do not engraft in the infarct zone or reduce infarct size have lead to controversy regarding the mechanism behind these promising results (8).

Despite these concerns, there is increasing evidence that a specific bone marrow constituent, the mesenchymal stem cell (MSC), has cardiac reparative properties. MSCs engraft (9), have the potential for myocyte differentiation (10), and release cytokines and growth factors that stimulate endogenous repair mechanisms (11, 12). Furthermore, MSCs have several properties that contribute to an ability to evade rejection (13–15). In this regard, they lack cell-surface B-7 costimulatory molecules (16, 17) and may also directly inhibit inflammatory responses (18). Therefore, MSCs may serve as an allogeneic graft, thereby avoiding the need for bone marrow harvesting from prospective recipients, an extraordinary therapeutic advantage for this cell type.

An additional consideration for the development of cellular therapeutics is the delivery approach. As cited above, intracoronary infusions of cells do not appear to infiltrate the MI zone or reduce MI size (3). Accordingly, the direct delivery of cells into the area of tissue necrosis may circumvent this potential limitation of cellular delivery.

Here, to address the hypothesis that MSCs reduce MI size and improve cardiac function, we conducted a randomized, investigator-blinded, placebo-controlled trial of MSCs in pigs after MI. Additional studies were performed by using cardiac MRI. The aims of this study were to (*i*) demonstrate that stem cells can be safely administered directly to damaged myocardium via injection catheter, (*ii*) test the efficacy of preprepared allogeneic MSCs as a cardiac cellular therapeutic strategy, and (*iii*) test the prediction that the mechanism of benefit is cellular engraftment, which results in cardiac myocyte regeneration, reduced infarct size, and improved cardiac function.

Methods

Animal Model. All animal studies were approved by the Institutional Animal Care and Use Committee and comply with the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication no. 80-23, revised 1985). Three different studies were conducted. First, we performed a randomized study of animals ($n = 14$) that underwent surgical induction of MI and instrumentation to measure left-ventricular (LV) performance and cardiac oxygen consumption. Second, a group of chronically instrumented animals ($n = 4$) were submitted to MI by balloon occlusion of the left anterior descending coronary artery (LAD), after recovery from surgery, so that measures of normal cardiac function could be obtained. Third, a group of animals ($n = 18$) were studied noninvasively with MRI after MI induced by balloon occlusion of the LAD.

Surgical Preparation. Female Yorkshire pigs underwent surgical instrumentation for subsequent noninvasive measurement of LV pressure and dimension and myocardial oxygen consumption (19, 20). The animals were instrumented, via a median sternotomy, with indwelling catheters in the descending aorta, right atrial appendage, and great cardiac vein. Endocardial ultrasound crystals (Sonometrics, Ontario, Canada) were inserted to measure short-axis dimension, and a pneumatic occluder was placed around the inferior vena cava for graded preload reduction to assess LV-pressure–dimension relations. A 4–5 mm flow probe (Transonics, Ithaca, NY) was placed around the mid-LAD to measure coronary volume flow. A solid-state miniature pressure transducer (P22, Konigsberg Instruments, Pasadena, CA) was placed in the LV apex for high-fidelity recordings of LV pressure. Additional pacing leads were

Abbreviations: Ees, ventricular elastance, slope of the end-systolic pressure–dimension relationship; LAD, left anterior descending coronary artery; LV, left ventricular; MI, myocardial infarction; MSC, mesenchymal stem cell; MVO_2, myocardial oxygen consumption per cardiac cycle; SW, stroke work.

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www.pnas.org/cgi/doi/10.1073/pnas.0504388102

Safety Study of Adult Mesenchymal Stem Cells (MSC) to Treat Acute Myocardial Infarction

This study is currently recruiting patients.
Verified by Osiris Therapeutics January 2006

Sponsored by: Osiris Therapeutics
Information provided by: Osiris Therapeutics
ClinicalTrials.gov Identifier: NCT00114452

Purpose

The purpose of the study is to determine whether adult stem cells [Provacel™(PUMP1)] are safe and possibly effective in the treatment of acute myocardial infarction (heart attack).

Condition	Intervention	Phase
Myocardial Infarction	Drug: Provacel	Phase I

MedlinePlus related topics: Heart Attack

Study Type: Interventional
Study Design: Treatment, Randomized, Double-Blind, Placebo Control, Parallel Assignment, Safety Study

Official Title: A Phase 1 Randomized, Double-Blind, Placebo-Controlled, Dose Escalation, Multicenter Study to Determine the Safety of Intravenous Ex-Vivo Cultured Adult Human Mesenchymal Stem Cells (Provacel) Following Acute Myocardial Infarction

Further study details as provided by Osiris Therapeutics:

Primary Outcomes: Comparison of treatment adverse event rates between the 0.5, 1.6 and 5.0 million mesenchymal stem cells per kilogram dose cohorts and placebo groups.
Expected Total Enrollment: 48

Study start: February 2005; Expected completion: December 2006
Last follow-up: September 2006; Data entry closure: October 2006

Summary of Potential Clinical Applications of MSCs

- Bone and cartilage repair
- Enhancement of auto/allogeneic HSC transplantation
- Correction of mesenchymal disorders
- As vehicles for gene therapy
- Repair of non-mesenchymal organs (CNS, CVS)
- Treatment or prevention of GVHD
- Tolerance induction to solid organs
- Tolerance induction to other hESC-derived cells
- Generation of HSCs from hESCs
- To facilitate engraftment of hESC-derived HSCs

University of Wisconsin
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AND PUBLIC HEALTH

Acknowledgement:

Parul Trivedi
Yi Ping Liu
James A Thomson

Trillium Fund for Multiple Myeloma Research